Colegio de Postgraduados

Artemisia cina ethanolic extract reduces the infection of *Haemonchus contortus* in lambs

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ABSTRACT

Objective: This study aimed to evaluate the anthelmintic efficacy of an ethanolic extract (Ac eth/ext) derived from leaves and stems of *Artemisia cina* in lambs artificially infected with *Haemonchus contortus*.

Design/Methodology/Approach: Three experimental groups of *H. contortus*-infected lambs (n=5) were assigned to the following treatments: (1) Ethanolic extract of *A. cina* (Ac eth/ext) at 4 mg/kg body weight (BW); (2) Albendazole at 5 mg/kg BW and (3) control group (5 mL of water). Treatments were administered orally as a single dose, and fecal egg count (FEC) along with ocular mucosa color were monitored for seven days.

Limitations/Implications: At the end of the study,all lambs were sacrificed, and the abomasum was extracted to recover adult parasites, which were subsequently counted and sexed.

Results: The Ac eth/ext group exhibited a significant 47% reduction in FEC compared to the control group ($p<0.05$). In contrast, albendazole treatment resulted in a 100% reduction in FEC values. The ethanolic extract also reduced the parasite burden by 30%, whereas albendazole led to a 5.31% reduction.

Findings/Conclusions: The ethanolic extract of *A. cina* (Ac eth/ext) could serve as a valuable complementary tool for the control of ovine haemonchosis. However, further research is necessary to identify the bioactive molecules responsible for its anthelmintic properties. Additionally, reducing the parasite burden in female worms may have a positive impact by lowering FEC values and subsequently decreasing pasture contamination.

Keywords: *Artemisia cina*, anthelmintic, *Haemonchus contortus*, ethanolic extract, fecal egg count.

INTRODUCTION

Haemonchus contortus is the most significant parasite affecting small ruminants raised under grazing systems (Lalramhluna *et al.*, 2020). This parasite induces severe clinical symptoms, including anemia, submandibular edema, and, in some cases, fatalities in young animals (Lambacher *et al.*, 2019). The primary control strategy for this nematodiasis relies on the regular administration of chemical anthelmintic drugs (AH) to reduce the parasitic burden in infected animals (Höglund *et al.*, 2020). However, the

widespread and continuous use of these drugs has led to the inevitable development of resistance in *H. contortus* populations (Fávero *et al.*, 2020). In response to this challenge, alternative control methods for haemonchosis and other parasitic nematodiases are being investigated as sustainable strategies distinct from conventional AH drug use. Among these, the application of medicinal plants has emerged as a promising approach, offering herbal-based therapeutic solutions with encouraging results (Szulc *et al.*, 2020). *Artemisia* is a plant genus within the Asteraceae family, widely recognized for its medicinal properties. Comprising 474 species, this genus is distributed globally, spanning polar regions to the tropics (Rustaiyan *et al.*, 2021). *Artemisia cina* Berg ex Poljakov, a shrub native to the eastern Caspian Sea region in Afghanistan, emits a distinctive aromatic odor, grows between 40 and 60 cm in height, and possesses erect, cottony stems. The leaves are small, with short petioles, and the upper leaves are sessile. This species thrives in semi-desert environments with extreme temperatures, favoring saline soils. Commonly referred to as cina or marine wormwood, *A. cina* has been traditionally employed as an antiparasitic remedy (Woerdenbag, 1997). Between the 1950s and 1970s, plant-based formulations derived from *A. cina* were used for deworming children in schools and childcare facilities. The active compound responsible for this effect, santonin, was initially formulated as tablets and later as flavored sweets, targeting the treatment of ascariasis, a highly prevalent parasitic infection (Zhang *et al.*, 2019). However, its clinical application was discontinued due to accumulated toxicity, with adverse effects observed at 60 mg in children and 200 mg in adults. Beyond its historical use, *A. cina* harbors bioactive compounds with diverse pharmacological properties. For instance, artemisinin, a molecule present in *A. cina* leaves, has been extensively recognized for its antimalarial activity and has been utilized for decades as an alternative treatment for malaria (Saitbaeva & Sidyakin, 1971). Additionally, *A. cina* exhibits significant anthelmintic potential. Ethanolic extracts of this plant have demonstrated notable *in vitro* anthelmintic activity, achieving up to an 80% efficacy against *H. contortus* infective larvae (L3) (Van Agtmael *et al.*, 1999). Interestingly, the anthelmintic properties of *A. cina* (commonly referred to as "worm killer") are attributed to phytoconstituents located in its aerial parts. Several bioactive compounds identified in these plant structures have exhibited efficacy against roundworms, pinworms, and amoebal infections in pigs and companion animals (Lans *et al.*, 2007). Beyond its *in vitro* effectiveness against *H. contortus*, *A. cina* has also demonstrated cestodicidal activity against *Moniezia expansa*, a tapeworm species infecting small ruminants (Bashtar *et al.*, 2011). The aim of this study was to evaluate the effects of orally administered ethanolic extracts of *A. cina* leaves on fecal egg count (FEC) and parasitic burden in lambs experimentally infected with *H. contortus* under controlled conditions.

MATERIALS AND METHODS

Location

This study was conducted at Laboratory 3 of the Multidisciplinary Research Unit, Faculty of Advanced Studies Cuautitlán – National Autonomous University of Mexico (UNAM). The animals were kept in paddocks at the Postgraduate Area of the same Faculty.

Plant material

Ten kilograms of *A. cina* Berg ex Poljakov leaf material at the pre-flowering stage were commercially obtained from Millennium Laboratories in Mexico City. The plants in this facility are cultivated in a greenhouse under controlled environmental conditions, including humidity (24.6%), temperature (24 °C), pH (8.7), and salinity (1.6%).

Obtaining plant extract

The plant material was dried at 60 °C for one week. The extract was obtained through organic extraction using ethanol for 24 hours. The resulting plant extract was then concentrated using a rotary evaporator (Heidolph Laborota 4000, Heidolph Instruments, Schwabach, Germany) under reduced pressure at 40 °C (Iqbal *et al.*, 2004). Finally, the extract was lyophilized using a Labconco FreeZone™ Freeze-Dry System (4.5 L).

Experimental animals

Fifteen three-month-old Hampshire male lambs, born nematode-free, were identified with metallic earrings and kept under full confinement conditions throughout the experiment. The animals were fed alfalfa (*Medicago sativa* L.) and had *ad libitum* access to water.

Artificial *H. contortus* infection

Lambs were randomly assigned to four groups and monitored for 14 days to ensure the absence of gastrointestinal nematode (GIN) eggs in their feces, thereby confirming that the experimental flock was free of parasitic infections. Each lamb was then intraruminally inoculated with 5,000 L3 larvae of H. contortus FESC strain. The animals were monitored weekly to determine their fecal egg count (FEC) starting from the third week post-infection. The lambs were included in the study once the flock reached a mean FEC of 5,000 eggs per gram.

Experimental procedure

Animals were randomly distributed into three groups of five animals each as follows: Group 1, *A. cina* ethanolic extract (Ac eth/ext), orally administered at a single dose of 4 mg/kg body weight (BW); Group 2, a single dose of albendazole (Albendaphorte 2.5% Co, Health and Animal Welfare Lab), orally administered at a dose of 5 mg/kg BW; Group 3, control, untreated. Fecal samples were collected daily directly from the rectum of each animal for seven days post-treatment to conduct the McMaster technique (Coles *et al.*, 1992). Additionally, a record of the FAMACHA© index was measured daily during the same period (Torres-Chable *et al.*, 2020).

Effect of *A. cina* ethanolic extract on the *H. contortus* faecal egg count reduction

Means of the faecal egg counts per group were estimated and compared with the control group. The results of this evaluation were expressed as the FEC reduction percentage, based on the following formula:

$$\%\ Reduction = 100 \times \frac{C - T}{C}$$

where C=FEC arithmetic mean in the control group and T=FEC arithmetic mean in the treated group.

Effect of *A. cina* ethanolic extract on reducing the *H. contortus* adult parasitic population in lambs at necropsy

The three experimental groups of lambs were slaughtered on day seven post-treatment under humane conditions, following the Norma Oficial Mexicana NOM-033-ZOO-1995, which regulates the humane slaughter of domestic and wild animals. The procedure for evaluating anthelmintic efficacy in ruminants, established by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Wood *et al.*, 1995), was followed.

The abomasum was removed, placed in individual plastic containers, and washed three times with preheated phosphate-buffered saline (PBS) at 30 °C. The contents were filtered through sieves with 1.25 mm and 0.25 mm diameters. The final filtrate was diluted with water to a total volume of 2000 mL and fixed with 10% formaldehyde. Next, 10 mL of this suspension was transferred onto a 60×15 mm plastic Petri dish to observe and recover adult parasites, which were then counted and sexed into two groups: males and females. This process was repeated until the entire initial volume was analyzed. The efficacy of the treatments was determined by comparing the mean number of *H. contortus* adult specimens recovered from each group with the control group, and the percentage reduction was calculated following the previously described formula (González-Garduño *et al.*, 2011).

Statistical analysis

The FEC and genus (male/female) data obtained from the three experimental groups were analyzed using the variance-ANOVA and the complementary Tukey tests to identify possible differences among groups using the Statgraphics program (Centurion XV®).

Ethical note

The lambs were managed by the guidelines of the Institutional Committee for the Care and Use of Experimental Animals of the FESC, University National Autonomous of Mexico (CICUAE-FESC-UNAM), protocol No. DC—2014-14.

RESULTS

Fecal egg count reduction

The results regarding the dynamics of fecal egg count (FEC) excretion in the three experimental groups are presented in Figure 1. Since the day with *A. cina* treatment, an increase in FEC values was observed for the three lamb-groups. Although, groups treated with albendazole and Ac eth/ext, this increase was significantly lower compared to the untreated control group.

Notably, from day 1 to day 6, the Ac eth/ext-treated group exhibited a continuous decline in FEC values. In contrast, the albendazole-treated group showed a significant reduction in FEC values from day 1, reaching zero on days 4, 5, and 6 of the experiment. The percentage reductions attributed to the anthelmintic effect of each treatment throughout the experiment are summarized in Figure 1. The mean FEC reduction percentages recorded during the experiment were 46.97% for the Ac eth/ext-treated group and 75.45% for the albendazole-treated group ($p<0.05$).

FAMACHA® values

The FAMACHA® values recorded throughout the experiment for the three experimental groups of lambs are presented in Table 1. Famacha score was observed since the day of treatment. Results showed that the untreated control group was consistently scored three in the FAMACHA® record, maintaining this value until the end of the experiment. In contrast, albendazole-treated group exhibited an improvement, with the FAMACHA® score decreasing to two, where it remained stable until the conclusion of the study. Similarly, in the Ac eth/ext-treated group, the FAMACHA® score remained at three until day four, after which it decreased to two on days five and six.

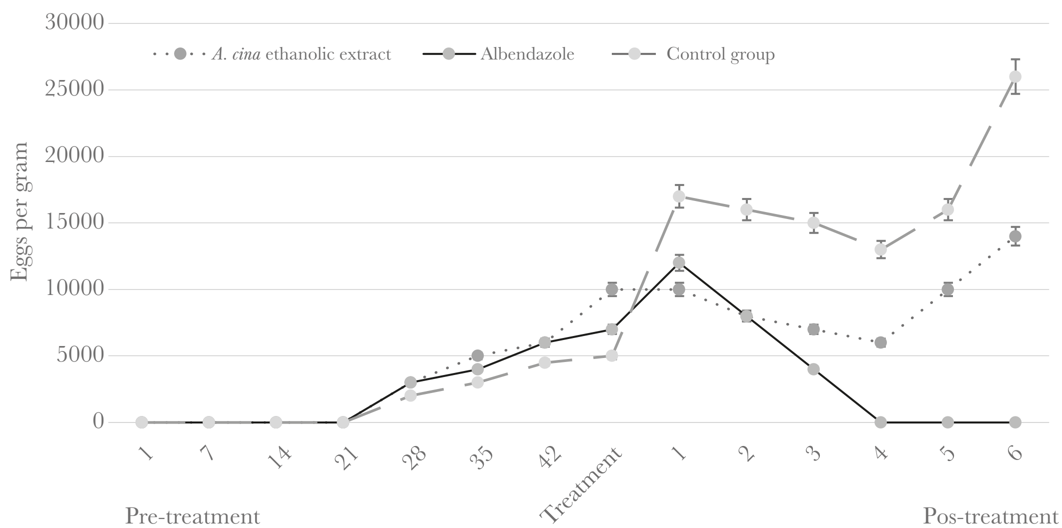

Figure 1. Records of the *Haemonchus contortus* EPG excreted by three groups of artificially infected lambs to determine the anthelmintic efficacy of *Artemisia cina* ethanolic extract.

Table 1. Ocular mucosa color (FAMACHA index) in artificially infected lambs with *Haemonchus contortus* treated with *Artemisia cina* ethanolic extract.

	FAMACHA index		
	Control group	***Artemisia cina* ethanolic extract**	**Albendazole**
Day 42	3 ± 0.5^a	3 ± 1^a	3 ± 1^a
Treatment	3 ± 1^a	3 ± 0.5^a	3 ± 1^a
Day 7 Post-treatment	3 ± 0.5^a	2 ± 1^b	2 ± 1^b

Superscripts show significant difference ($p<0.05$).

Anthelmintic effect of *A. cina* in lambs

Table 2 summarizes the data on the mean numbers of *H. contortus* males and females recovered at necropsy, along with the anthelmintic efficacies of Ac eth/ext and albendazole. The Ac eth/ext treatment resulted in a 30% reduction in total parasitic burden, whereas albendazole demonstrated only a 5.31% reduction. The efficacy of Ac eth/ext against male and female parasites did not show any statistically significant differences between them.

DISCUSSION

Reduction of FEC values attributed to the effect of *A. cina* ethanolic extract

The results obtained in the present study provide clear evidence of the anthelmintic effect of Ac eth/ext, which was observed from the first record (day 1) and persisted until the last record (day 6), with efficacy ranging from 37.5% to 53.8% compared to the control group. In this regard, it is noteworthy that, to date, no previous records exist regarding the anthelmintic effect of *A. cina* organic extracts in reducing *H. contortus* FEC excretion in lambs. Therefore, this study could be considered the first report documenting the anthelmintic properties of this plant against *H. contortus* in lambs. On the other hand, a study conducted in Cairo, Egypt, evaluated the anthelmintic effect of an *A. cina* hydroalcoholic extract under *in vitro* conditions, demonstrating that the extract induced structural damage to the scolex and other tissues of the cestode *Moniezia expansa* as observed through electron microscopy. Additionally, the authors reported that oral administration of 2 g of *A. cina* crude extract per sheep eliminated *M. expansa* fecal egg counts by day 9 post-treatment (Bashtar *et al.*, 2011). It is also noteworthy that, during the first three days post-treatment, the FEC reduction effect of albendazole was lower than expected, considering that some studies report that albendazole reaches its peak plasma concentration (approximately 1.2 μg/mL) around eight hours post-administration (Evrard *et al.*, 2002). However, the subsequent total reduction in FEC excretion observed in the following days confirms that albendazole remains a highly effective compound in controlling *H. contortus* infections in lambs. Nevertheless, it is crucial to acknowledge that the recurrent use of any anthelmintic drug in farm animals inevitably leads to the development of anthelmintic resistance, which in turn rapidly diminishes their efficacy (Kellerová *et al.*, 2020).

Table 2. Number, prolificity, length, male:female ratio of *Haemonchus contortus* females recovered from artificially infected lambs with *H. contortus* treated with *Artemisia cina* ethanolic extract.

Treatment	Eggs eliminated by adults of *H. contortus*	Number of *H. contortus* adult females*	Female length (mm) of *Haemonchus contortus*	No. of male adults of *H. contortus**	*H. contortus* lenght of adult males (mm)	Male: female ratio
Ac eth/ ext	51,134±544[b]	365±12[a]	2.09±0.2[a]	306.25±23[a]	1.4±0.2[a]	0.83:1[a]
Control	37,319±251[c]	496.25±15[b]	1.9±0.3[b]	463±27[b]	1.47±0.2[a]	0.93:1[a]
Albendazole	531±31[d]	276±15[c]	2.07±0.2[a]	632.5±17[c]	1.48±0.2[a]	2.2:1[c]

**Haemonchus contortus* adult stages were collected from the abomasum of infected lambs and kept in incubation with RPMI medium to be counted and measured. Ac eth/ext=*Artemisia cina* ethanolic extract; superscripts show a significant difference ($p<0.05$).

Effect of *A. cina* ethanolic extract on the FAMACHA records

The FAMACHA® records in animals treated with Ac eth/ext showed an improvement on days 3, 5, and 6 of the experiment compared to the control group. This suggests that the Ac eth/ext-treated group likely had a lower parasitic burden, leading to reduced blood loss and, consequently, the maintenance of packed cell volume (PCV) values. This observation was further supported by the post-mortem reduction in parasitic burden in the Ac eth/ext-treated lambs compared to the control group. In the case of albendazole, a substantial reduction in FAMACHA® scores was observed from day 2, and this reduction persisted throughout the experiment until its conclusion.

These findings support the strategic deworming approach, reinforcing the principle that not all animals in a flock should be dewormed, but only those that require it. Furthermore, the results provide compelling evidence that Ac eth/ext represents a valuable complementary tool for minimizing the unnecessary use of anthelmintics, thereby indirectly contributing to reducing the spread of anthelmintic resistance.

Effect of administration of *A. cina* ethanolic extract on reducing the parasitic burden at necropsy in lambs

The reduction in the number of adult parasites recovered at necropsy in the Ac eth/ext-treated group, compared to the nematodes recovered from the untreated control group, indicates that although the reduction was moderate (30%), it can still be considered significant. This is particularly relevant when considering that albendazole demonstrated an efficacy closer to 100% (5.3%). The high efficacy of albendazole observed in this study suggests that the *H. contortus* strain used in the experiment may have developed anthelmintic resistance. However, to confirm this assumption, additional parameters such as body condition, packed cell volume (PCV), and the FAMACHA® index should be analyzed in conjunction with the Fecal Egg Count Reduction Test (FECRT) (Wood *et al.*, 2002; Höglund *et al.*, 2020). Similarly, albendazole has been widely reported in Mexico as a reference anthelmintic; however, its continuous use has led to documented cases of resistance, as reported by various authors listed in Table 3. The frequent and prolonged use of anthelmintic drugs has similarly contributed to resistance in sheep and goats. Evidence presented in Table 3 suggests that anthelmintic resistance is a growing concern in Latin America (Torres-Acosta *et al.*, 2012).

Regarding the anthelmintic efficacy of albendazole against gastrointestinal nematodes (GIN), several reports highlight a worrisome increase in resistance to this and most commercially available anthelmintic drugs across multiple countries (Table 3).

Due to the limited information regarding the use of *A. cina* extracts as potential anthelmintics in ruminants, further studies are necessary to enhance the anthelmintic efficacy observed with Ac eth/ext. For instance, research should explore the effects of increasing extract concentrations or administering higher doses. Additionally, it is crucial to design bio-guided experiments to identify the specific compounds responsible for the anthelmintic activity, ultimately paving the way for the development of a novel, alternative, and sustainable method for controlling sheep haemonchosis (Delgado-Nuñez *et al.*, 2020). Since ancient times, plants and natural products have been regarded as valuable resources

Table 3. Recent records about albendazole anthelmintic resistance in ruminant parasitic nematodes in different countries.

Country	Resistance (%)	Parasitic genera	Reference
Bangladesh	25-47	*H, O, Tr*	Dey *et al.*, 2020
Sudan	73.5-90.2	*Hc, Tr, C*	Mohammedsalih *et al.*, 2020
Brazil	100	*C, Hc, Tr*	Ramos *et al.* 2020
Iran	71	Natural infection	Ebrahimi *et al.*, 2020
Egypt	13.16	*Hc*	Aboelhadid *et al.*, 2020
France and Italy	0-5	Natural infection	Chartier *et al.*, 2020
Indonesia	16.19	*Tr, As*	Kholik *et al.*, 2019
Sudan	18	*Hc*	Mohammedsalih *et al.*, 2019
Mexico	83	*C, Tr*	Mondragón-Ancelmo *et al.*, 2019
Malang Regency	7.77	Natural infection	Sasangko *et al.*, 2019
Brazil	34	Natural infection	Nagata *et al.*, 2019
Mozambique	52.9	*Hc*	Atanásio-Nhacumbe *et al.*, 2019
Pakistan	54	Natural infection	Muhammad *et al.*, 2019
Brazil	1-38	Natural infection	De Fátima Lela Pererira *et al.*, 2019
Jordan	60	Natural infection	Hayajneh *et al.*, 2019
Brazil	100	Natural infection	Costa *et al.*, 2019
Brazil	100	*Co, Hc, Oes, Tr*	Ramos *et al.*, 2018
Brazil	95	*Hc, Tr, Oe, Tri*	Flávida da Silva *et al.*, 2018
Australia	100	*Hc, Tr, Os, C*	Rashid *et al.*, 2018
Madhya Pradesh	28	Natural infection	Shakya *et al.*, 2018
Iraq	100	*Tr, Mar, Tri, Ne*	Dyary, 2018
India	33	*Hc*	Sahoo *et al.*, 2018
Slovakia	13.2-30.8	Natural infection	Babják *et al.*, 2018

Hc: *Haemonchus contortus*, *Tr*: *Trichostrongylus axei*, *Oe*: *Oesophagostomum colombianum*, *Tri*: *Trichuris ovis*, *Os*: *Ostertagia ostertagi*, *Mar*: *Marshallagia* spp., *Ne*: *Nematodirus* spp.

for human and veterinary medicine. This traditional knowledge is essential for exploring phytochemical and pharmacological properties, providing scientific foundations for their sustainable use (Dassou *et al.*, 2020). The anthelmintic properties of *A. cina* against intestinal parasites in children, particularly Ascaris lumbricoides, were first documented in 1950 in China. The active compound santonin, a sesquiterpene, was identified as the primary agent responsible for its antiparasitic activity. For many years, santonin was one of the most widely used agents for the control of intestinal parasitic diseases in China (Zhang *et al.*, 2019). The anthelmintic efficacy of various *Artemisia* species against *H. contortus* has been demonstrated in several studies (Table 4).

High variability in *Artemisia* spp. anthelmintic activity against *H. contortus*

One of the most practical approaches to integrating plant-based anthelmintic treatments into flock management is by incorporating plant material into the diet as a nutritional supplement. For instance, the addition of *Artemisia absinthium* L. leaves to the

Table 4. Recent reports concerning the anthelmintic effect of *Artemisia* spp. against *Haemonchus contortus in vitro* and *in vivo.*

***Artemisia* spp.**	**Part of the plant**	**Extract**	**Experimental condition**	**Anthelmintic efficacy***	**Author**
A. absinthium	Stems	Dietary supplement	*In vivo*	45%	Váradyová *et al.*, 2017
A. absinthium	Leaves	Ethanolic Aqueous	*In vivo*	100% 80%	Alam *et al.*, 2016
A. absinthium	Leaves	Dietary supplement	*In vivo*	100%	Valderrábano *et al.*, 2010
A. absinthium	Leaves and stems	Aqueous	*In vivo*	No differences	Worku *et al.*, 2009
A. absinthium	Leaves	Aqueous Ethanolic	*In vitro*	80.49% 90.46%	Tariq *et al.*, 2009
A. absinthium	Leaves	Dietary supplement	*In vitro* *In vivo*	99% No differences	Mravcakova *et al.*, 2020
A. absinthium	Stems	Methanolic Aqueous	*In vitro*	100%	Váradyová *et al.*, 2018
A. annua *A. absinthium*	Leaves	Aqueous Ethanolic Essential oil	*In vivo*	No significant effects	Squires *et al.*, 2011
A. annua	Leaves	Hydroalcoholic	*In vitro*	93.22%	Sprenger *et al.*, 2016
A. annua	Leaves	Aqueous	*In vivo*	No differences	Cala *et al.*, 2014
A. brevifolia	Whole plant	Aqueous Methanol	*In vivo*	67.2%	Iqbal *et al.*, 2004
A. campestris	Leaves	Aqueous Ethanolic	*In vitro*	50%	Akkari *et al.*, 2014
A. campestris	Aerial parts	Essential oil	*In vitro*	100%	Abidi *et al.*, 2018
A. campestris L.	Leaves	Aqueous	*In vitro*	<50%	Boyko *et al.*, 2019
A. cina	Leaves	Homeopathic	*In vivo*	69%	Higuera-Piedrahita *et al.*, 2020
A. cina	Leaves and stems	Ethanolic	*In vivo*	47%	Present study
Artemisia afra	Leaves	Aqueous	*In vitro*	100%	Molefe *et al.*, 2012
A. herba-alba	Leaves	Methanolic	*In vitro*-IEH	67%	Ahmed *et al.*, 2020
A. herba-alba	Leaves	Dietary supplement	*In vivo*	100%	Idris *et al.*, 1982
A. lancea	Leaves	Essential oil	*In vitro*	99%	Zhu *et al.*, 2013
A. parviflora *A. sieversiana*	Leaves	Methanolic	*In vitro*	100% 90%	Irum *et al.*, 2017
A. vestita *A. maritima*	Leaves	Aqueous	*In vivo*	87.2% 84.5%	Irum *et al.*, 2015
A. vulgaris L.	Leaves	Essential oil	*In vitro*	No differences	Malik *et al.*, 2019
A. tridentata	Leaves	Aqueous	*In vitro*	96.30%	Luck-Montero *et al.*, 2018
A. vulgaris	Aerial parts	Aqueous Ethanolic	*In vitro*	100% 100%	Karim *et al.*, 2019

* The anthelmintic efficiency value described in the table was taken as the highest reported in each paper.

diet of a Rasa Aragonesa lamb flock in Zaragoza, Spain, resulted in a 100% reduction in *H. contortus* fecal egg count (FEC) (Valderrábano *et al.*, 2010). In a similar study, Váradyová *et al.* (2018) reported a 45% FEC reduction in a Vallachian lamb flock in the Slovak Republic following the voluntary consumption of *A. absinthium* pellets. However, another study conducted in the Slovak Republic, in which *A. absinthium* was administered as part of a mixed plant diet, did not demonstrate a significant FEC reduction (Mravčáková *et al.*, 2020). The high variability observed in the anthelmintic response across different studies conducted in various agroecological zones may be explained by the biological activity of plants, which is influenced by numerous biotic and abiotic factors. These factors, primarily associated with the environment where the plants grow, subject plants to various stresses that may modify their morphological, physiological, and biochemical activities (Sardharal & Mehta, 2018).

Recent studies have identified several key factors regulating plant biosynthesis, including light exposure, plant hormones, temperature, and saline and drought stress. Notably, a study demonstrated that abiotic stressors, such as ultraviolet B irradiation and the presence of phytohormones, stimulated artemisinin accumulation in *Artemisia annua* (Ma *et al.*, 2020). Regarding the anthelmintic activity of Artemisia species extracted using different organic solvents under in vitro conditions, researchers have reported high variability in efficacy, ranging from 50% (*e.g.*, *Artemisia campestris* L.) to close to 100% (*e.g.*, *A. absinthium*, *A. annua*, and *A. campestris*) (Sprenger *et al.*, 2016; Váradyová *et al.*, 2018; Abidi *et al.*, 2018; Mravčáková *et al.*, 2020). Furthermore, the inclusion of Artemisia leaves in the diet as a nutritional supplement for small ruminants has been associated with FEC reductions ranging from 45% to 100% (Table 4). Similarly, when organic extracts of Artemisia species were administered orally, reported FEC reductions ranged from 67.2% (*e.g.*, *A. brevifolia*) to 100% (*e.g.*, *A. absinthium*) (Table 4). It is important to consider that secondary metabolites responsible for anthelmintic activity, such as flavones, flavonoids, alkaloids, coumarins, sesquiterpenes, and santonins, are typically extracted using organic solvents, due to their specific polarity (Muhammad *et al.*, 2021). Consequently, aqueous extractions are not generally expected to exhibit strong anthelmintic properties. However, a study by Alam *et al.* (2016) reported an 80% FEC reduction in *H. contortus*-infected calves treated with an aqueous extract of *A. absinthium*. Additionally, essential oils extracted from various Artemisia species have been found to contain monoterpenoids, including camphor, 1,8-cineole, camphene, and α-pinene, which are associated with antioxidant and antimicrobial properties (Sharopov *et al.*, 2020). Notably, monoterpenes, such as thymol (reagent grade), have also been linked to antiparasitic activity against other helminth species of veterinary importance, demonstrating high efficacy in rodent models (Mirza *et al.*, 2020).

CONCLUSIONS

Artemisia cina ethanolic extract demonstrated a 47% reduction in fecal egg count (FEC) in *Haemonchus contortus*-infected lambs under controlled conditions. This treatment also resulted in a 30% decrease in parasitic burden. *Artemisia cina* ethanolic extract represents a valuable tool for the control of haemonchosis in lambs, and further studies are necessary to assess its efficacy within an integrated control system, combining alternative control strategies to enhance flock health from a sustainable perspective. Additionally, the identification of bioactive metabolites responsible for the anthelmintic activity should be pursued to evaluate their potential application in the control of gastrointestinal parasitic nematodes.

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